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GBV-C/Hepatitis G Virus (HGV) RNA Load in Immunodeficient Individuals and in Immunocompetent Individuals

J.-J. Lefrère,[1]* C. Férec,[2] F. Roudot-Thoraval,[3] P. Loiseau,[4] J.F. Cantaloube,[5] P. Biagini,[5] M. Mariotti,[1] G. LeGac,[2] and B. Mercier[2]

[1]*Institut National de la Transfusion Sanguine, Paris, France*
[2]*Etablissement de Transfusion Sanguine et CHU de Brest, France*
[3]*Service de Santé Publique, Hôpital Henri-Mondor, Créteil, France*
[4]*Etablissement de Transfusion Sanguine de l'Assistance Publique-Hôpitaux de Paris, Paris, France*
[5]*Etablissement de Transfusion Sanguine d'Alpes-Provence, France*

The aim of this study was to establish the mean plasma GBV-C/ hepatitis G virus (HGV) RNA load in groups of GBV-C/HGV-infected individuals with varied immune status and to determine the most frequent patterns of evolution of the plasma GBV-C/HGV RNA load over time during the natural history of infection. The mean plasma GBV-C/HGV RNA load observed was, from the lowest to the highest: 5.21 log in immunodepressed multiply-transfused patients, 6.45 log in HIV-positive individuals, 6.66 log in immunocompetent multiply-transfused patients, and 6.71 log in blood donors. The difference was significant between the four groups ($P < 0.0001$). The most frequent pattern of evolution of the plasma GBV-C/HGV RNA load was as follows: after the primary GBV-C/HGV infection, the viral load was elevated from the onset; then, a high, persistent and relatively steady viral RNA level was the rule; and when it occurred, the loss of viremia was not preceded by a decrease before recovery from GBV-C/HGV infection. ***J. Med. Virol. 59:32–37, 1999.***

KEY WORDS: GBV-C/hepatitis G virus RNA; immunodeficiency; polymerase chain reaction; viral load

INTRODUCTION

The RNA virus termed GBV-C/hepatitis G virus (HGV) and belonging to the *Flaviridae* family was described in 1995 [Muerhoff et al., 1995; Simons et al., 1995a,b; Leary et al., 1996; Linnen et al., 1996]. Three years later, despite a large number of investigations, the clinical significance of the GBV-C/HGV infection remains unresolved. Cases of acute or chronic hepatitis [Muerhoff et al., 1995; Simons et al., 1995a,b; Heringlake et al., 1996; Leary et al., 1996; Linne et al., 1996; Alter et al., 1997] have been linked to this infection, but the role of the GBV-C/HGV in these conditions has been rebutted subsequently [Alter et al., 1996; Lefrère et al., 1997; Loiseau et al., 1997]. The most recent studies confirm that the GBV-C/HGV infection appears totally symptomless in all carriers whatever their immune status [Alter et al., 1996; Kudo et al., 1996; Lefrère et al., 1996, 1997; Loiseau et al., 1997].

The epidemiology and the routes of transmission of the virus are largely known: GBV-C/HGV is bloodborne and sexually-transmitted, and individuals at high risk of infection for such viruses have a high prevalence of GBV-C/HGV markers [Jarvis et al., 1996; Linnen et al., 1996, Stark et al., 1996; Nubling et al., 1996; Wang et al., 1996]. The natural history of the GBV-C/HGV infection is also known: once acquired, the GBV-C/HGV infection generally tends to persist for several years in immunocompetent as in immunodeficient individuals [Kudo et al., 1996; Lefrère et al., 1997]. After this period of symptomless viremia, the majority of viral RNA carriers clears the virus over time, with the appearance of an antibody to the envelop protein E2 (anti-E2 antibody) [Tacke et al., 1997]. The presence of such an antibody characterizes seroconversion and reflects recovery from GBV-C/HGV infection.

No specific study has been published on the plasma GBV-C/HGV RNA load nor on its evolution during the natural history of infection. Furthermore, the eventual influence of the immune status on the level of GBV-C/HGV RNA load in GBV-C/HGV-infected individuals is unknown. For this reason, a quantitative reverse transcription (RT) TaqMan polymerase chain reaction (PCR) assay was developed, permitting the determina-

*Correspondence to: J.-J. Lefrère, Institut National de la Transfusion Sanguine, 53 boulevard Diderot, 75012 Paris, France. E-mail: lefrere@worldnet.fr

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tion of the GBV-C/HGV RNA load in plasma. The GBV-C/HGV RNA load in groups of individuals with varied immune status and selected previously as positive for GBV-C/HGV RNA by a qualitative RT-PCR assay [Lefrère et al., 1997, 1999a,b; Loiseau et al., 1997] was also evaluated. Thus, the present study had two aims: first, to establish the mean plasma GBV-C/HGV RNA load according to the immune status of the GBV-C/HGV-infected individuals and according to several epidemiological characteristics; and second, to determine the most frequent patterns of evolution of the plasma GBV-C/HGV RNA load over time during the natural history of GBV-C/HGV infection.

MATERIALS AND METHODS

Patients

Cross-sectional study. Four groups of GBV-C/HGV RNA-positive individuals with varied immune status were formed:

Group A included 32 individuals diagnosed as positive for GBV-C/HGV RNA by screening of a cohort of immunodepressed multiply-transfused patients treated by intravenous immunoglobulin as long-term replacement therapy [Lefrère et al., 1999b]; five were affected with a common variable immune deficiency and had received, over their entire life, only intravenous immunoglobulins as blood products; and 27 were bone marrow transplant patients, who had received both intravenous immunoglobulin and multiple cellular products (including packed red cells and platelets). There were 22 men and 10 women, with a mean age of 28 years (range, 5–61).

Group B included 28 human immunodeficiency virus (HIV)-1-infected individuals diagnosed as positive for GBV-C/HGV RNA by the screening of a cohort of HIV-positive individuals [Lefrère et al., 1999a]. Risk factors of HIV infection were homosexual men (n = 15), intravenous drug addicts (n = 6), and heterosexual contacts (n = 7). There were 20 men and 8 women, with a mean age of 30 years (range, 20–46). At the time of sampling, all belonged to stage A according to the Center for Disease Control (CDC) 1993 criteria and did not receive any antiviral therapy. Their mean CD4+ T cell count was 590/mm^3 (range, 180–1110).

Group C included 11 immunocompetent multiply-transfused individuals diagnosed as positive for GBV-C/HGV RNA by screening of a cohort [Loiseau et al., 1997] of patients affected with hemoglobinopathy (thalassaemia major or sickle cell disease) and multiply-transfused with packed red cells. There were eight men and three women, with a mean age of 18 years (range, 5–33).

Group D included 64 individuals diagnosed as positive for GBV-C/HGV RNA by a prevalence study [Loiseau et al., 1997; Cantaloube et al., 1999] carried out in two cohorts of volunteer and unpaid blood donors (from blood transfusion centers of the Paris area and of Marseille). There were 38 men and 26 women, with a mean age of 37 years (range, 20–60).

Longitudinal study. This study was based on the sequential samples collected annually during the follow-up of two cohorts of GBV-C/HGV-RNA-positive individuals:

The first cohort included the 28 HIV-positive individuals of group B; all had been diagnosed as chronic carriers of GBV-C/HGV RNA by a previous screening [Lefrère et al., 1999b]. Twenty of these 28 individuals had a well-defined duration of HIV infection identified by an HIV-negative assay within the six months preceding the first HIV-positive assay. The 28 HIV-positive individuals were shown to carry GBV-C/HGV RNA over a mean period of 7.6 years (range, 3–12). Among these, 24 were GBV-C/HGV RNA-positive since their first visit, while 4 acquired GBV-C/HGV during the follow-up period. At the end of the study period, 27 of the 28 individuals were still positive for GBV-C/HGV RNA. The mean CD4+ T cell count of the 28 HIV-positive individuals at the first sample collected annually positive for GBV-C/HGV RNA and at the last sample positive for GBV-C/HGV RNA was 568 ±200 and 405 ± 199/mm^3 ($P < 0.001$). At the end of the follow-up period, 8 HIV-positive individuals had been treated with antiviral drugs over a mean period of 16 months (range, 0–54); 3 had entered into the stage B/C of the CDC classification.

The second cohort included the 11 immunocompetent multiply-transfused patients of group C; all had been diagnosed as chronic carriers of GBV-C/HGV RNA by screening [Lefrère et al., 1997]. These 11 patients were shown to carry GBV-C/HGV RNA over a mean period of 3.5 years (range, 1–8). Among them, five were GBV-C/HGV RNA-positive since their first visit, while six acquired GBV-C/HGV during the follow-up period. At the end of the study period, 8 of the 11 patients were still positive for GBV-C/HGV RNA.

Test Methods

The quantification of plasma GBV-C/HGV RNA was carried out according to a TaqMan PCR assay [Burlot et al., submitted]. Briefly, nucleic acids were extracted from 200 μl plasma samples using the Viral Nucleic Acid Extraction Kit (Boehringer Mannheim, Germany), according to the manufacturer's instructions. Three microliters of the samples contained extracted nucleic acids were used for carrying out the reverse transcription reactions in accordance with manufacturer's instructions (Boehringer Mannheim, Germany) and using hexamers. Amplification was performed in a 50 μl reaction mixture containing 10 mM Tris-HCl, pH 8.3, 50 mM Kcl, 5 mM $MgCl_2$, 200 μM dNTPs (dUTP instead of dTTP), 15 pmoles of each GBV-C/HGV primer, 7.5 pmoles of the GBV-C/HGV-labeled probe, 1 unit of Amperase, 2 units of Taq Gold polymerase, and 5 μl of the reverse transcription preparation. The amplifications and detections were performed in a Perkin Elmer 7700 apparatus according to optimized conditions. After an incubation of 2 minutes at 50°C for digestion of the product with Amperase, a 10 minute incubation at 95°C allowed the activation of the Taq Gold polymerase and the denaturation of the nucleic acids.

Fifty cycles of 30 seconds at 95°C and 30 seconds at 60°C were carried out, allowing the amplification-detection of GBV-C/HGV genomas. Quantification standards corresponding to GBV-C/HGV RNA-positive samples in which the GBV-C/HGV RNA load was quantitated by a branched DNA assay signal amplification (bDNA assay, Chiron Corporation, Nucleic Acids Systems, Emeryville, CA) were used. The results were expressed in log 10 (log) of equivalents per mL of plasma.

Serum alanine aminotransferase (ALT) level was determined by an automated method (Vitros 950, Johnson and Johnson, Rochester, NY) and expressed in IU/l. The upper limit of normal ALT level of the laboratory was 40 IU/L.

The detection of serum HCV antibodies was undertaken by a third generation enzyme-linked immunosorbent assays (Elisa) (EIA 3.0 HCV, Ortho Diagnostic Systems, Roissy, France; Monolisa HCV, Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France), with validation of each positive result by a third generation recombinant immunoblotting assay (Riba 3.0, Ortho Diagnostic Systems). In the immunodepressed patients (common variable immune deficiency patients and bone marrow transplant patients), in whom only genomic amplification was reliable to diagnose an HCV infection, the serum HCV RNA detection was carried out by a RT-PCR, using a protocol validated by a multicentre quality control [Lefrère et al., 1994].

Methodology of the Study

Cross-sectional study. The mean GBV-C/HGV RNA load was compared between the four groups; a relationship between the GBV-C/HGV RNA load and sex, age, status for HCV infections and ALT level was studied. In group B, a relationship between the GBV-C/HGV RNA load and the CD4+ T cell count, and between the GBV-C/HGV RNA load and the HIV RNA load was studied.

Longitudinal study. In the two cohorts followed up over time, the quantification of plasma GBV-C/HGV RNA was carried out by testing frozen serum samples which had been collected annually between the first and the last samples found positive for GBV-C/HGV RNA through the previous screening studies [Lefrère et al., 1997, 1999b]. The mean GBV-C/HGV RNA load was calculated over the whole follow-up period.

Statistical Methods

Results were expressed as mean ± 1 SD or as percentages. Chi-square test or Fisher exact test was used for the comparison of categorical data. Kruskal-Wallis test was used to compare independent qualitative data; in case of a significant global difference, multiple pairwise comparisons were performed, taking into account the number of comparisons to keep an overall alpha value of 0.05. Wilcoxon test was used for paired data. A linear regression analysis was performed to test the relationship between two quantitative variables. All analyses were carried out by means of BMDP statistical software [BMDP, 1990]. Differences were considered significant at $P < 0.05$.

RESULTS

Cross-Sectional Study

The mean plasma GBV-C/HGV RNA load observed in the four studied groups (Fig. 1) was from the lowest to the highest: 5.21 log (± 1.23; range, 1.75–6.92) in immunodepressed multiply-transfused patients (group A), 6.45 log (± 0.84; range, 3.81–7.67) in HIV-positive individuals (group B), 6.66 log (± 0.39; range, 5.81–7.13) in immunocompetent multiply-transfused patients (group C), and 6.71 log (± 0.82; range, 3.94–7.78) in blood donors (group D), respectively. The difference was significant between the four groups ($P < 0.0001$), and this difference was due to the value observed in group A, which was significantly lower than that observed in each of the three other groups ($P < 0.05$).

In the whole population of this cross-sectional study, no significant relationship was found between the level of plasma GBV-C/HGV RNA load and sex (6.23 and 6.36 log in males and females, respectively), age, status for HCV infection (6.07 and 6.31 log in HCV-infected and in HCV-negative individuals, respectively), or ALT level (6.0 and 6.33 log in individuals with an elevated ALT level and in individuals with a normal ALT level, respectively). In the group B individuals, no significant relationship was found between the plasma GBV-C/HGV RNA load and the CD4+ T cell count ($r = -0.07$), and between the plasma GBV-C/HGV RNA load and the plasma HIV RNA load ($r = -0.15$).

Longitudinal Study

The mean plasma GBV-C/HGV RNA load of the immunocompetent multiply-transfused cohort and of the HIV-positive cohort over the follow-up period is shown in figures 2A and 2B. If the two cohorts are considered as a whole, 26 of 39 (66.7%) individuals had a plasma GBV-C/HGV RNA load higher than 5 log over the follow-up period. One individual (2.6%) belonging to group B had a plasma GBV-C/HGV RNA load lower than 5 log over the whole follow-up period. The lowest and the highest values of plasma GBV-C/HGV RNA load observed during the study period were 2.2 and 8.3 log, respectively.

The most frequent pattern of evolution of the plasma GBV-C/HGV RNA load was as follows: after primary GBV-C/HGV infection, the viral load was increased from the onset, as observed in the ten individuals of the two groups with a known date of GBV-C/HGV infection (appearance of a detectable plasma GBV-C/HGV RNA after a period of negative PCR results), then a high, persistent and relatively steady viral RNA level was the rule; when it occurred, the loss of viremia was not preceded by a decrease in the years preceding recovery from GBV-C/HGV infection. Such a loss of GBV-C/HGV RNA was observed in four patients of the two cohorts (among them, however, a 2 log-decrease was observed in a case in the latter months preceding the seroconversion to GBV-C/HGV).

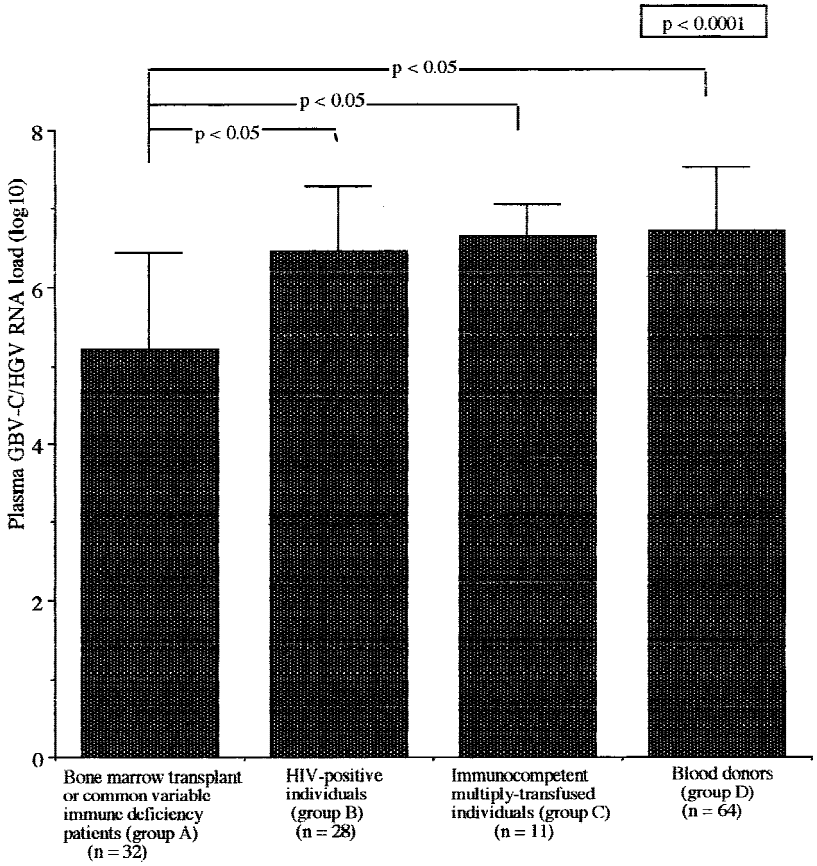

Fig. 1. Mean plasma GBV-C/HGV RNA load observed in the four studied groups.

During the study period, an elevated ALT level was observed on at least one control in nine patients of the two cohorts (28.2%). Among these nine individuals, eight belonged to the HIV-positive group (six were HCV-infected and one was positive for hepatitis B surface antigen). The ninth patient belonged to the group of immunocompetent multiply-transfused patients, and his elevated ALT level was observed only in one of the ten samples collected over the study period. Among the ten individuals of the two groups in whom the date of GBV-C/HGV infection could be documented, only one had an elevated serum ALT level on the first sample positive for GBV-C/HGV RNA.

DISCUSSION

The main findings of the present study were that the large majority of GBV-C/HGV-infected individuals presented a high and steady viral RNA level over time and that this level was inversely correlated to the immune status. Studies published previously, based on semiquantitation of GBV-C/HGV RNA load by end-point dilution in nested PCR [Mazuko et al., 1996; Shimizu et al., 1997], or on quantitation by home-made PCR assay [Alter et al., 1997] or by branched-DNA assay [Lau et al., 1997; Martinot et al., 1997; Pessoa et al., 1997], have shown that the plasma GBV-C/HGV RNA load could reach values at least ten-fold higher than that of the HCV RNA load. Our follow-up study indicates that the plasma viral RNA load is persistently high during the natural history of GBV-C/HGV infection.

The fact that the most immunodeficient GBV-C/HGV-infected individuals of our study had a level lower than the GBV-C/HGV-infected individuals with no or mild immunosuppression (such as our HIV-positive individuals, who still had a high CD4+ T cell count) is surprising and difficult to explain. There are at least two hypotheses: first, all group A immunodepressed individuals of the study being treated chronically by intravenous immunoglobulin as long-term replacement therapy, an effect of anti-GBV-C/HGV antibodies acquired passively from the intravenous immunoglobulin administration may not be excluded: indeed, due to the high prevalence of plasma anti-E2 antibody in blood donors [Lefrère et al., 1999b], intravenous immunoglobulin batches are rich in such an antibody [Morris et al., 1998], and probably in other anti-GBV-C/HGV antibodies, and may contribute, by neutralising a part of the infectious virus, to decrease the circulating GBV-C/HGV RNA load of the recipients.

Second, the lower GBV-C/HGV RNA load in immunodeficient patients could be linked to an alteration of the site of replication of the virus. This site is still unknown, but must be particularly active to cause such a persistently high viremia. The fact that, in case of se-

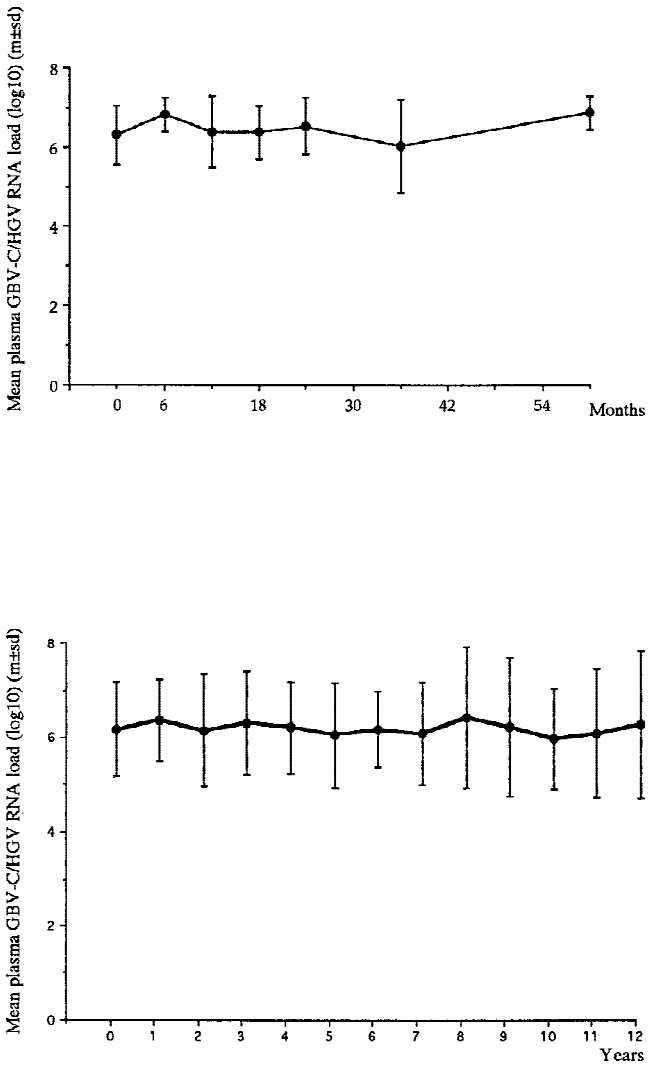

Fig. 2. **A:** Mean plasma GBV-C/HGV RNA load of the immunocompetent multiply-transfused individual cohort over the follow-up period. **B:** Mean plasma GBV-C/HGV RNA load of the HIV-positive individual cohort over the follow-up period.

vere impairement of the immune function, the circulating viral load is lower could suggest either that the site of viral replication is the immune system itself, or that, to be maximal, the replication of the virus needs a fully functional immune system.

Furthermore, if the immune status influences the level of the GBV-C/HGV RNA load, it does not seem to play a role in the duration of carrying the virus. Indeed, lengthy carriage of GBV-C/HGV may be observed in immunocompetent individuals as in immunodeficient individuals. The mechanisms responsible for the sudden loss of GBV-C/HGV RNA and for the appearance of anti-E2 antibody are still unresolved: why does this GBV-C/HGV RNA loss occur only after a variable number of years following the first infectious contact of the virus with the organism? Why does the recovery from GBV-C/HGV infection occur at a given time and not at another? The mechanisms allowing a recovery to take place after several years of persistent carrying of the virus are unknown. Research on this topic could identify elements about the mechanisms which allow viruses to persist or to be eliminated from the organism by the host's immune system.

In our study, other than the immune status, no parameter influenced the level of the GBV-C/HGV RNA load, including the age of GBV-C/HGV-infected individuals. However, a previous study found a significantly higher level of GBV-C/HGV RNA in patients 40 years of age or younger than in patients older than 40 years of age [Martinot et al., 1997].

Furthermore, our results confirm the increasing data in the literature indicating that the GBV-C/HGV is not responsible for hepatitis. Most of the GBV-C/HGV RNA-positive individuals in the current study had no biochemical evidence of liver damage. Moreover, no clinical evidence of a disease potentially linked to the GBV-C/HGV infection was observed during the follow-up of the patients, who were shown to carry GBV-C/HGV RNA for a long period. However, the contrast between the lack of disease association and the high and persistent viremia in GBV-C/HGV infection is surprising and the virus must remain under investigation.

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